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RETROVIRUS-HOST INTERACTIONS. THE MOUSE MAMMARY TUMOR VIRUS MODEL

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Summary Mouse mammary tumor virus (MMTV) is a retrovirus which can induce mammary carcinomas in mice late in life by activation of proto-oncogenes after integration in their vicinity. Surprisingly, it requires a functional immune system to achieve efficient infection of the mammary gland. This requirement became clear when it was discovered that it has developed strategies to exploit the immune response. Instead of escaping immune detection, it induces a vigorous polyclonal T-B interaction which is required to induce a chronic infection. This is achieved by activating and then infecting antigen presenting cells (B cells), expressing a superantigen on their cell surface and triggering unlimited help by the large number of superantigen-specific T cells. The end result of this strong T-B interaction is the proliferation and differentiation of the infected B cells leading to their long term survival.

Kew words: retrovirus, mammary tumor virus, superantigen, T-B interaction, cancer

First descriptions of mammary tumors in mice were found over 100 years ago[1]. From this first description to the observation of maternal transmission of the susceptibility to mammary tumor development via milk it took nearly 100 years[2-4]. It became clear that this milk factor was responsible for the majority of mammary carcinomas in many different mouse strains. From this observation to the general acceptance of the retroviral nature of this tumor-causing milk factor again 20 years passed[5-7]. One of the reasons for the scepticism was the finding that tumor-free mouse strains were found to be infected with such retroviruses in the mammary gland and that some mouse strains did develop tumors even when fed on mammary tumor free mothers. The presence and role of endogenous viruses was not known yet (see below). Thereafter, mouse mammary tumor virus (MMTV) was a key tool in cancer research until retroviruses carrying their own oncogenes were discovered. Many of the classical experiments are summarized in several older and recent reviews and will not be included here in detail[8-10]. It became quiet about this virus because the readouts for most of the experiments were too long (up to one year for cancer development after injection of MMTV into adult mice) and the isolation of large quantities of virus was difficult (mostly virus was isolated from mouse milk or at much lower levels from tumor cell culture supernatants).

Several reports indicated a role of a functional immune system in promoting the infection[11-13]. Especially the paper in this series by Tsubura et al.[12] indicated that a functional immune system was required to transfer the infection from the original target of infection, the lymphocyte, to the mammary gland.

In 1991 MMTV came back to the center of research when it was found simultaneously by several groups that MMTV carries the answers to an old immunological puzzle: In the seventies,

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Festenstein had discovered antigens which allowed mixed lymphocyte reactions between major histocompatibility complex (MHC) compatible mouse strains which were even stronger than the classical MHC alloresponses. Due to the allocation of the name "major" to the MHC, these molecules were called minor lymphocyte stimulat-ing (Mls) antigens[14]. A first crucial discovery was that mice expressing such Mls loci, clonally deleted T cells bearing specific T cell receptor (TCR) Vβ elements during thymic maturation and that T cells reacting to such Mls antigens had the very same TCR Vβ elements on their cell surface (see chapter on superantigens)[15, 16]. In the 7 key papers solving this puzzle about 20 years after its discovery it became clear that these powerful antigens were encoded in an open reading frame encoded by the long terminal repeat of mouse mammary tumor virus (MMTV) which was already described but not understood previously by retrovirologists[17-23]. Within this open reading frame in the 3' long terminal repeat of MMTV a particular type of protein was encoded: a superantigen (SAg)[22-24].

Superantigens

Classically, antigens recognized by T cell receptors (TCR) are small peptides bound in the peptide binding groove of MHC molecules. The frequencies of T cells recognizing such peptides in the context of self MHC molecules are in the order of 10^{-4} to 10^{-6}. Several of the important peptide binding polymorphic amino acids in the TCR are located in the third complementarity determining region which is formed during the rearrangement of the V, D and J elements during rearrangement of the TCR. SAgs on the other hand interact with a much

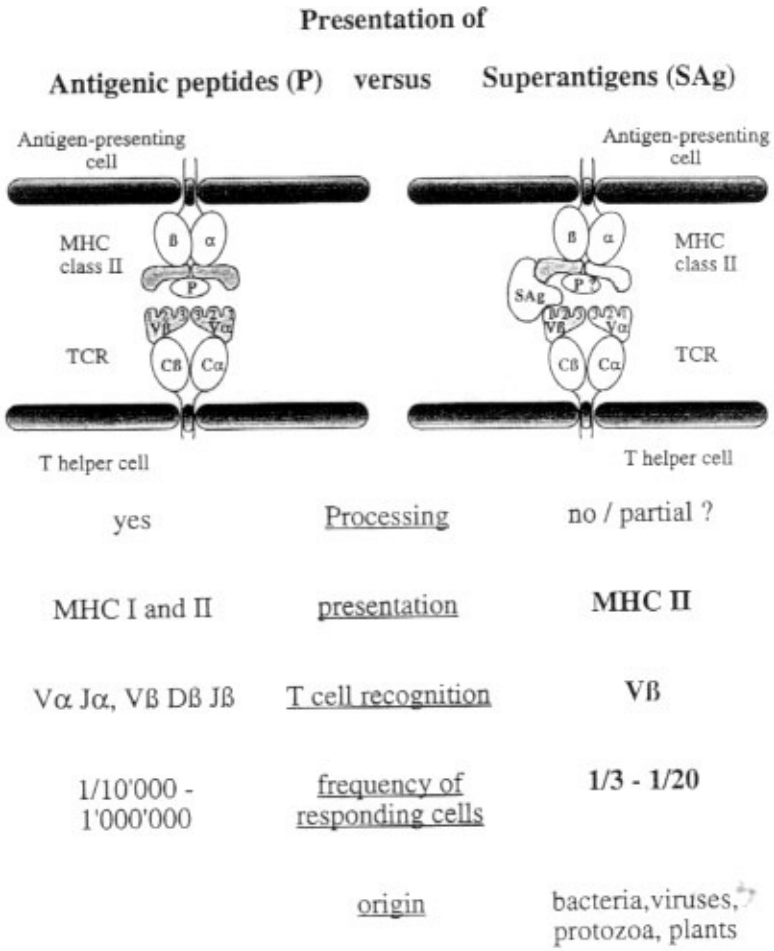

Fig. 1.– Comparison of antigen- (left) and superantigen (right)-presentation

higher percentage of T cells. It became clear that SAgs are not just T cell mitogens but interact with T cells expressing one or several of the TCR Vβ elements. They bind to the much less polymorphic lateral side of the TCR and of the MHC class II molecules (see Figure 1). Since there is a limited number of these Vβ elements (25 in mice) it is easy to explain the high frequency of SAg-reactive cells (in the order of 5-30%). Contrary to classical antigens such SAgs are not processed to small peptides but function as entire molecules or as partially cleaved molecules. Therefore, SAgs trigger a polyclonal T cell response by T cells which usually would have millions of different peptide antigen speci-ficities. The only requirement being the expression of a particular TCR Vβ element. The availability of anti-TCR Vβ specific antibodies allows to follow the SAg-reactive T cells *in vivo* and *in vitro*.

One might ask what purpose such SAgs have. The immune system has developed refined strategies to recognize and eliminate foreign substances. It can detect single amino acid differences between foreign and self proteins. SAgs can activate T cells with thousands of different fine specificities. The answer lies in the origin of SAgs: all the so far described SAgs are produced by microbes. Over 20 exotoxins of bacteria have been described with SAg activity. Bacterial SAgs are secreted by many different strains of bacteria such as Staphylococci, Streptococci, Mycoplasma and Yersinia[25, 26]. They have also been found in viruses (rabies)[27], and retroviruses (mouse mammary tumor virus, MMTV).

Bacterial SAgs are exotoxins which are secreted by the bacteria. The rabies SAg is a nucleocapsid protein which is carried in the viral capsid. MMTV encodes a SAg which is only produced after reverse transcription and integration of the retroviral cDNA into the host cell's genome.

Although it is unlikely that the different bacteria have developed SAgs with quite different primary amino acid structure without reason, there is no clear understanding of the advantage they bring to the bacteria. One of the best supported hypotheses is an inhibition of the host immune response against the bacteria by an over stimulation of the immune system with production of excess cytokines and lymphocyte interactions which is not directed against the invading agent. Experimental evidence for this interpretation is, however, not too strong[28]. The roles of MMTV SAgs in the retroviral life cycle became clear recently and are outlined in more detail below.

The three phases of MMTV infection

The three phases of MMTV infection are summarized in Figure 2.

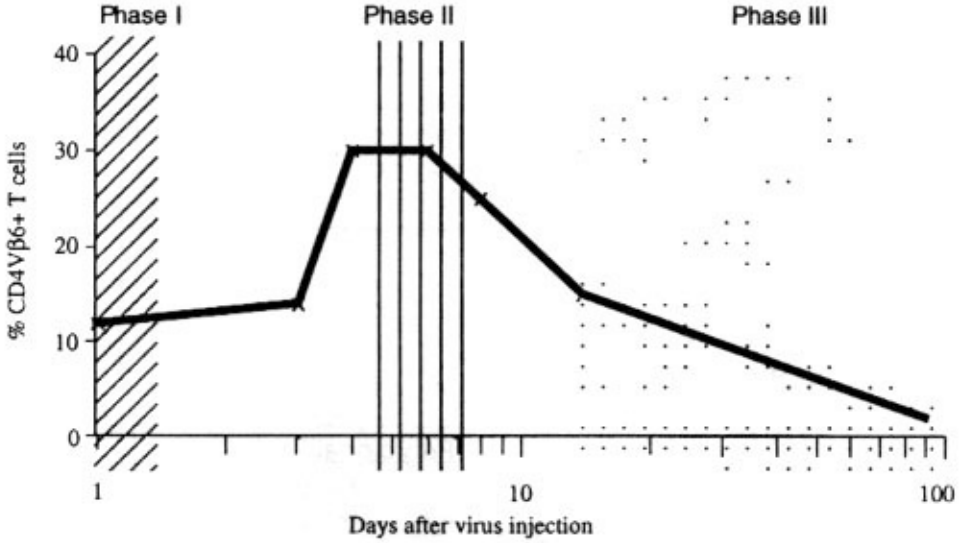

Fig. 2.– The three phases of the MMTV-host interaction. When injected subcutaneously at day 0 within 1 day B cells are activated polyclonally (phase I). Around day 4-6 the peak response of the SAg-reactive T cells and the infected B cells is observed (phase II). From day 6 on efficient virus neutralization is observed and maintained life long. SAg-reactive T cells get deleted (phase III).

Infection-activation (Phase I)

The way MMTV infects its target cells is still unclear. What became clear recently was that one of the first targets of MMTV infection are the B lymphocytes[29, 30]. There is, however, some indirect evidence for SAg presentation by dendritic cells (see below)[31].

Recently we showed that MMTV activates the B cells but not T cells within hours of virus injection[32]. The SAg-reactive T cells became activated only later after about 36 hours. When high virus doses were used, up to 90% of B cells became activated within 18 hours of virus injection. Among these activated B cells only few were infected. Interestingly, retroviruses require activated lymphocytes for infection to occur[33]. Most retroviruses need cells in cell cycle to allow integration of the reverse transcribed viral genome into the host DNA. HIV can infect T cells which are not in cycle but they need to be activated[34]. For MMTV it is not known what is required but the early virus-dependent activation of their target of infection leads the way. Possibly only the few B cells which enter cell cycle after virus binding can get productively infected.

SAg-response (Phase II)

Once viral gene products are expressed (most likely after viral integration into the genome) the SAg can be presented on the surface of the B cells in the context of MHC class II molecules. It is this step that is so important for the establishment of a productive infection (see below). SAg-reactive T cells which make up 5-35% of the T cell repertoire interact with the SAg-presenting infected B cells and help them to divide (increasing the numbers of infected B cells at least 1000 fold within 6 days of infection), differentiate and

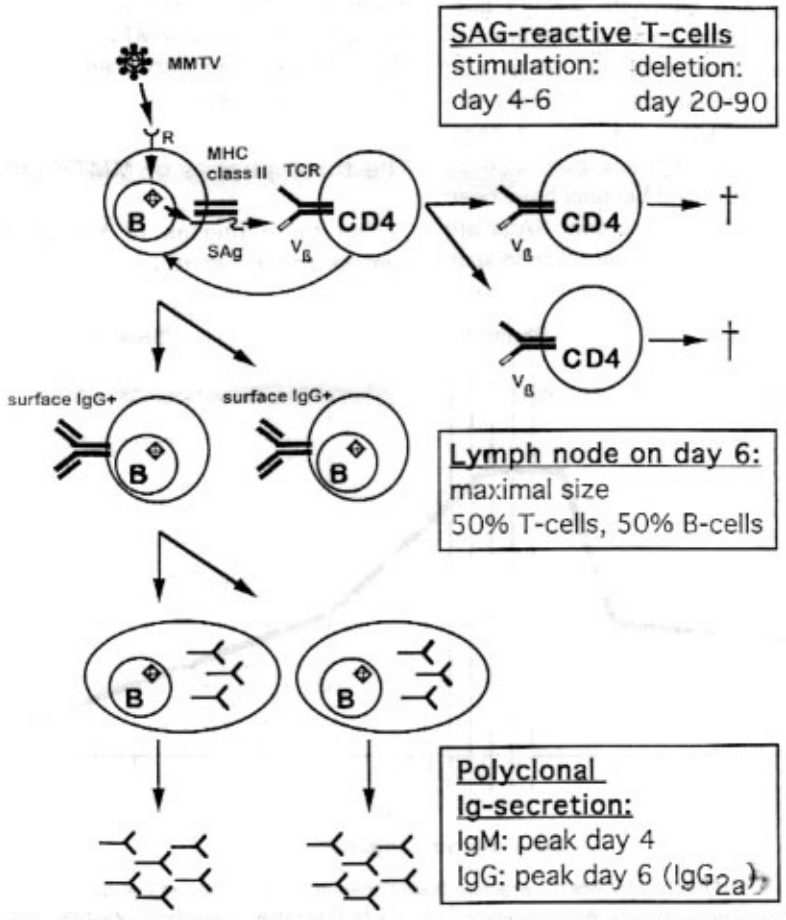

Fig. 3.– Interactions between MMTV and the immune system. TCR: T cell receptor; R: receptor for MMTV; ⊠ integrated provirus.

to become long lived B cells. First T cell activation is seen around day 2 after virus injection. The SAg response is summarized in Figure 3. Each division of an infected B cell will increase the number of infected cells and differentiation of an infected B cell into a long lived B cell will increase the chance of the virus to fulfil its life cycle.

Chronic infection, virus neutralization and mammary gland infection (Phase III)

Infection of neonatal or adult mice with MMTV leads to life long infection in 100% of cases in susceptible mouse strains. Shortly after infection, an efficient neutralizing antibody response can be measured which, however, is incapable of eliminating the infection[8, 35]. For the moment it is not clear whether this immune response just occurs too late, helps the host prevent re-infection or viral spread or whether it serves the retrovirus to better survive in the host. Interestingly this neutralizing antibody response is SAg-dependent meaning that in the presence of a SAg response the neutralization is much stronger than in its absence[35]. It is not clear whether there is a preferential infection of MMTV-specific B cells which then are amplified by the SAg response or whether the viral amplification due to the SAg response leads to higher levels of viral protein and hence to induction of a neutralizing response. Later, both CD4 and CD8 T cells are infected by unknown mechanisms and finally the virus is brought to the mammary gland by these infected lymphocytes. Since virus spreads between these lymphocyte subsets it has been impossible to find which subset was responsible for the transfer to the mammary gland[12, 13]. So far no clear evidence for a role of a cytotoxic response against MMTV has been found.

Importance of the SAg-response for infection

Several studies have shown that the SAg-presentation step is key in the life cycle of MMTV[30, 36, 37]. Absence of SAg-reactive T cells leads to lack of the SAg response and highly decreased probability to achieve infection of the mammary gland. After one to five generations the virus is lost from the mouse strain it originally infected[30, 37]. Similar results were obtained in mouse strains expressing MHC class II molecules which cannot present MMTV SAgs (I-A^a) or in mice lacking B cells or MHC class II molecules[38,39]. In additional experiments it was shown that recombinant MMTV's which express a SAg with a point mutation rendering it non functional select rare mutants re-expressing a functional SAg[40]. These were clear indications about a crucial role of the SAg response in the maintenance of MMTV infection in the mouse population.

Exploitation instead of evasion from the immune system

Many viruses have found strategies to evade from the immune response such as pox viruses which use a large portion of their genome to evade the host immune response[41]. MMTV is the first described virus which uses a more offensive strategy to guarantee its survival. It induces a very strong immune response which has as its main purpose the increase in the number and survival time of the infected B cells. Without the action of its SAg the virus cannot survive in the mouse population.

MMTV and mammary carcinomas

MMTV is responsible for the majority of mammary carcinomas in mice. Inbred mouse strains were derived with high, intermediate or low mammary tumor incidence. In strains with high incidence, up to 100% of mice had mammary carcinomas by the age of 1 year[42]. Forced breeding increased the tumor incidence dramatically. This is best illustrated in the review by Hageman et al.[42] where many breeding experiments with different MMTV's are summarized. For example, 100% of force bred BALB/c mice fostered by C3H mothers developed mammary tumors after 6-7 months whereas the frequency was 84% at 11 months in virgin mice. These results clearly reflect the role of hormones in MMTV biology. In the long terminal repeats of MMTV hormone responsive elements are found which increase transcription of viral DNA[43].

Contrary to other oncogenic retroviruses, MMTV does not carry an oncogene in its genome. One of the steps involved in oncogenesis is the

integration of the viral genome close to host proto-oncogenes. Through the action of the 3'LTR promotor and enhancer genes located in the vicinity of the integration site can be expressed. Frequent integrations close to such proto-oncogenes were described, and so far at least 6 of these int genes (int-1 to-6) have been characterized. These proto-oncogenes were initially named integration (int) genes and functions of some of them have been determined[44, 47]. Recently, the nomenclature for some of them was changed to Wnt (a hybrid between wingless and int) (for review see[48]).

Not all MMTVs which efficiently infect a particular mouse strain lead to high incidences of mammary carcinomas. For example, MMTV (C3H) induces mammary carcinomas within 1 year in nearly 100% of BALB/c mice (reviewed in[42]) whereas MMTV(SW) promotes cancer development in only very rare occasions[49]. This low level of tumor incidence was found despite the high amounts of MMTV in the milk of the infected mothers. Analysing the few known MMTV's lead to the observation that MMTV's with very potent SAgs such as MMTV(SW) and MMTV(C4) lead to strong immune responses and low tumor incidences whereas MMTV's with weaker SAgs such as MMTV (C3H), MMTV (SIM), and MMTV (SHN) lead to weaker SAg responses, slower deletion kinetics and much higher and quicker tumor onsets[49, 52]. The reasons for these differences are not clear since the SAg-reactive T cells are most likely not the T cells implicated in the regulation of tumor growth.

In rare cases MMTV-induced tumors other than mammary carcinomas have been described. Most were found in T lymphocytes. Originally, it was surprising that the lymphocytes which are the first targets of infection are much less susceptible to tumor induction. When it was shown that all these lymphomas had a deletion spanning part of the 3' LTR it became clear that lymphocytes are most likely protected from tumorigenesis by MMTV. The regions deleted in all of these tumors contained the 3' end of SAg as well as many gene regulation elements[53-55]. Although the molecular reasons for this protective effect are poorly understood, tumorigenesis in the lymphoid compartment before viral transmission would represent an end point in the viral life cycle.

A particular case was reported with endogenous Mtv29. This Mtv is found in SJL mice which develop so called reticular cell lymphomas after 1 year of age. It was shown that these tumors are in fact follicular B cell lymphomas due to overexpression of the Mtv29 SAg[56]. The tumors grew due to secretion of cytokines by the continuously activated SAg-reactive T cells. Two groups made very similar observations; one describing TCR Vβ17[57], the other TCR Vβ16 expressing T cells[58-60] as the responsible cytokine secretors. In this system overexpression of the SAg in old mice leads to tumor formation and overcomes neonatal tolerance; a situation which could also be regarded as a case of autoimmunity.

An indirect effect of Mtv SAgs on tumor formation was observed with polyoma-induced tumors. Mouse strains expressing Mtv7 are protected from these tumors on the H-2^k background due to clonal deletion of T cells expressing TCR Vβ6. $V\beta6^+$ $CD8^-$ T cells are crucial for elimination of polyoma-induced tumors; in their absence polyomas appear[61].

The life cycle of MMTV

A summary of the MMTV life cycle is given in Figure 4. High titers of MMTV are produced in the milk of infected female mice. The babies are infected after drinking milk via their intestine[62]. Infection through this route is possible during the first two weeks of life, before the stomach acidifies. The virus enters through the dome region which covers the Peyer's patches and infects B cells in the Peyer's patches within days of birth. So far it is unclear whether dendritic cells are infected before B cell infection occurs. Peak SAg responses are found in the Peyer's patches within 8-9 days after birth. The B cells already produce large amounts of antibodies at this stage. After MMTV infection both extrafollicular and follicular B cell maturation is observed (see below). Several days later the virus-infected cells become detectable in other lymphoid organs. B cells are the main infected lymphocyte population within the first week of infection. By unknown mechanisms virus infects thereafter other lymphoid subsets. Throughout the life of the mouse a small fraction of B, CD4 as well as CD8 T cells are infected with MMTV and they are found

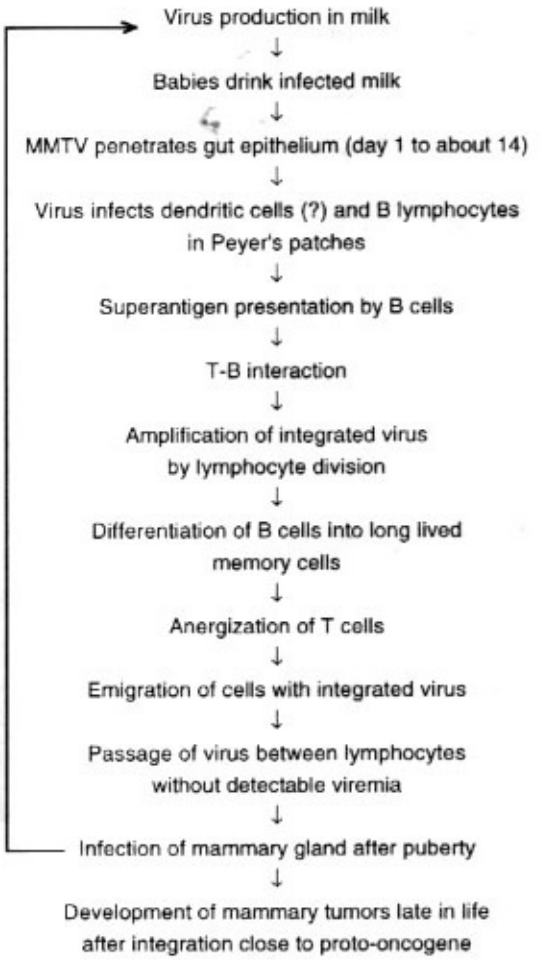

Fig. 4.– Life cycle of MMTV

in all lymphoid and in several non-lymphoid organs. Mammary gland infection has been detected around puberty but it has not been looked carefully yet at which exact time point infection occurs.

How to use MMTV to investigate the immune response

Knowledge of the virus host interactions allows to utilize viral infection as a tool to investigate the immune system. From the several aspects of this I will introduce only one in more detail: The SAg-driven T-B interaction. Other aspects are the ability to follow the infected B cells which carry a new integrated provirus through the body. This marker allows the observation of the fate and migration patterns of B cells which were involved in a primary T-B interaction.

T-B interactions

As already outlined above, T cells recognizing an MMTV SAg are activated, divide and differentiate into long-lived T cells. Contrary to current belief, few of these cells die early after infection. Most survive the initial SAg-response and are slowly lost; most likely due to their inability to enter cell cycle. If this were true their deletion would give an indication about their half life. There would be (at least) two populations, one dying with a half life of about a month, the other slower. Newly generated potential SAg-reactive T cells are deleted in the thymus and do not complicate these observations. We recently asked the question whether B cells differentiation induced by a SAg is comparable or different to classical antigen-responses. For the comparison we used nitrophenol-haptenated chicken gammaglobulin (NP-CGG) in Alum and with or without pertussis adjuvant in comparison with MMTV infection[31, 63]. Both primary responses were of a similar amplitude, following similar kinetics. The responses were surprisingly similar. In the case of MMTV an unlimited help by SAg-reactive T cells was available whereas in the primary NP-CGG response this was not expected. Contrary to our expectations we observed similar kinetics of detectable T cell responses. In both cases priming was observed in close contact with interdigitating cells (dendritic cells) around day two. A day later, T-B interaction was observed in the outer cortex. This was surprising for the MMTV response since we had assumed that B cells were the major subset of infected cells. The only major difference between the two responses was the appearance of the first germinal centers. In the case of the classical antigen they appeared in parallel with the extrafollicular B cell differentiation whereas in the case of MMTV it appeared around one week later, when the extrafollicular response had already terminated. The separation of the two responses allowed it now to investigate the role of the extrafollicular and the follicular response in the formation of an antigen-specific immune response and to estimate the half life of the presumably short lived plasmablasts. The results on B and T cell differentiation are summarized in figure 5.

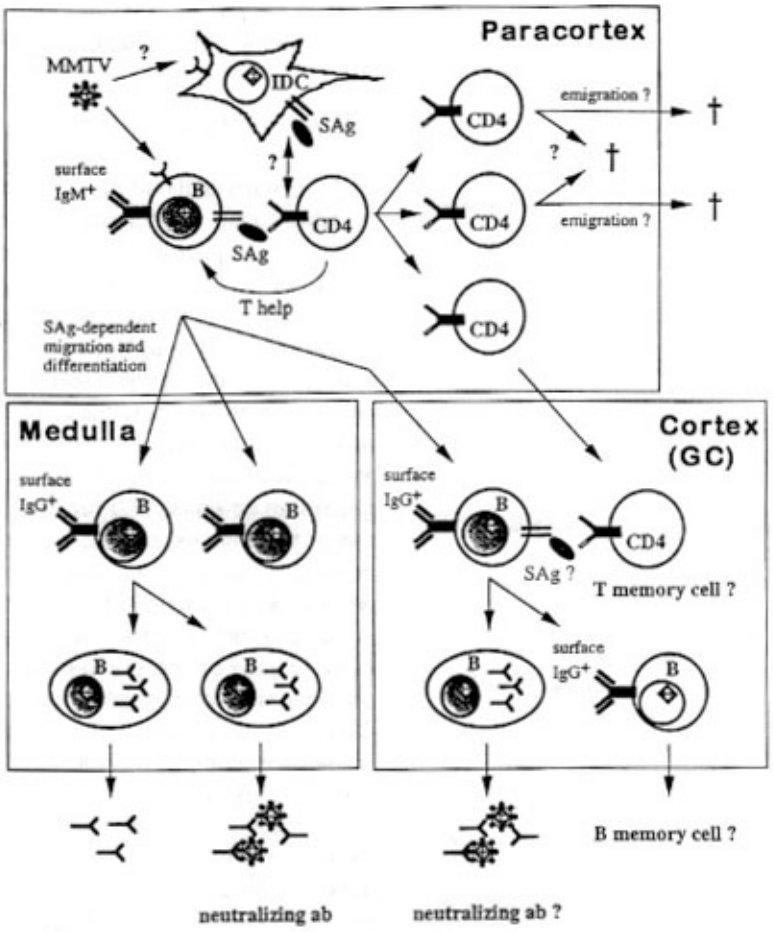

Fig. 5.– MMTV-SAg-induced T-B interaction in the draining lymph node. GC: Germinal center. This figure was reproduced from reference 63 with permission.

Outlook

The virus host interaction between MMTV and its host, the mouse, is becoming better and better understood. This knowledge serves as a very useful tool to investigate basic questions about immunology and retrovirology. Roles for similar viruses in human cancer have repeatedly been put forward and the acquired results with MMTV will help to clarify this issue.

Resumen

Interacciones retrovirus-huésped. El modelo del virus del tumor mamario en el ratón

El virus del tumor murino del ratón (MMTV) es un retrovirus capaz de inducir carcinomas mamarios tarde en la vida del ratón a través de la activación de proto-oncogenes cercanos a su integración en el genoma celular. Sorprenden-temente, este retrovirus necesita un sistema inmune funcionante para conseguir una eficiente infección de la glándula mamaria. Este requerimiento se entendió cuando se descubrió que el virus desarrolla estrategias para explotar la respuesta inmune. En lugar de escapar al reconocimiento inmune, el virus induce una vigorosa interacción policlonal T-B la cual es necesaria para inducir una infección crónica. Esto se obtiene activando y luego infectando células presentadoras de antígeno (células B) las que expresan un superantígeno en su membrana desencadenando ayuda ilimitada de un gran número de células T superantígeno-específicas. El resultado final de esta fuerte interacción T-B es la proliferación y diferenciación de las células B infectadas asegurándoles una larga sobrevida.

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